Sexually Transmitted Infections

Editorials

Use of nucleic acid amplification tests in investigating child sexual abuse

Because of the medical-legal implications, the identification of a sexually transmitted disease, especially *Chlamydia trachomatis* and *Neisseria gonorrhoeae,* in a prepubertal child requires the use of methods with the highest specificity. Diagnosis of *C trachomatis* infection in this setting has been based on isolation of the organism in tissue culture. Culture requires careful specimen collection and stringent transport conditions with maintenance of the cold chain and requires 48–72 hours to perform. In addition, culture methods for *C trachomatis* are not standardised and there can be significant variation in performance from laboratory to laboratory.[1] Obtaining appropriate specimens requires a vaginal swab in children. Similarly, the definitive diagnosis of gonorrhoea has been based on culture of *N gonorrhoeae,* which entails isolation on selective media. Although culture of *N gonorrhoeae* is relatively inexpensive and highly sensitive, it is logistically complicated. As with the collection of specimens for culture of chlamydia, detection of *N gonorrhoeae* also requires vaginal swabs in children. The invasive nature of the specimens needed creates additional trauma for victims of sexual assault.

The introduction of nucleic acid amplification tests (NAAs) has been the most important advance in the field of chlamydia diagnostics since tissue culture replaced inoculation of eggs for culture and isolation of *C trachomatis* from clinical specimens. Because nucleic acid amplification is exquisitely sensitive, theoretically capable of detecting as little as a single gene copy, and highly specific, it offers the opportunity to use non-invasive sampling—that is, urine. There are now four NAAs approved by the US Food and Drug Administration (FDA) for the detection of *C trachomatis* in clinical specimens: polymerase chain reaction (PCR), Amplicor *Chlamydia trachomatis* test (Roche Molecular Diagnostics), ligase chain reaction (LCR), LCx *Chlamydia trachomatis* Assay (Abbott Diagnostics), transcription mediated amplification (TMA) (GenProbe), and strand displacement amplification (SDA) (ProbeTec, Becton Dickson). PCR, LCR, and SDA are DNA amplification tests; TMA is an RNA amplification assay. Currently NAAs are approved for cervical swabs from women, urethral swabs from men, and urine from men and women. None of these tests are approved or recommended by the manufacturers for rectal specimens from adults and they are not approved for rectogenital specimens from children.

NAAs are more sensitive than culture for detection of *C trachomatis* in genital specimens in adults, detecting an additional 25–30% over culture.[2] Multiple studies in adults have demonstrated sensitivities of >80–100% compared with 65–88% for culture, while maintaining high specificities (95–100%).[2] Although all these assays are approved for use with urine from women, the sensitivities are lower than those of endocervical swabs.[3–8] Practically all of these studies have been done in high prevalence populations (3–15%). However, despite high sensitivities and specificities, false positive and false negative results can occur. False negatives due to inhibitors of DNA polymerase are more of a problem than false positives because of Amplicon carryover. Inhibitors appear to be more frequent in cervical specimens. LCR appears to be less susceptible to inhibitors than PCR. Of note, SDA is the only currently available assay that includes inhibition controls.

There is less experience with the use of NAAs for detection of *N gonorrhoeae* in clinical specimens. Unlike *C trachomatis*, culture of *N gonorrhoeae* is well standardised and widely available. However, there have always been concerns about the loss of viability during transport to the laboratory. The following NAAs now have FDA approval for detection of *N gonorrhoeae* in genital swabs and urine from men and women—LCR, PCR, TMA, and SDA. Unlike the experience with NAAs for detection of *C trachomatis,* the performance of these assays has not been dramatically better than standard culture methods for detection of *N gonorrhoeae*.[3–6 8]

The use of urine for the detection of *C trachomatis* and *N gonorrhoeae* in children who are being evaluated for suspected sexual abuse is very attractive. However, are NAA tests of sufficient sensitivity and, most importantly, specificity, to be used in non-invasive specimens from prepubertal girls? Although one can probably extrapolate from the performance of these tests with urine specimens from adult women to adolescent women, one may not be able to do so for younger girls. Most of the evaluations in adults have been done in high prevalence populations (>5%). Performance in low prevalence populations has not been as good, especially for detection of *N gonorrhoeae*.[6] The prevalence of infection with *C trachomatis* and *N gonorrhoeae* in prepubertal girls who are suspected victims of sexual abuse has generally been ⩽2%. Everett *et al* reported prevalences of genital infection with *C trachomatis* and *N gonorrhoeae* of 1.3 and 2%, respectively in 2973 girls evaluated for sexual abuse over a 16 year period.[9] Data on use of NAAs with vaginal specimens from prepubertal girls are very limited. A Canadian study compared PCR with culture of vaginal wash specimens for detection of *C trachomatis* from 25 prepubertal girls.[10] Four of 25 (16%) samples were positive by PCR and were confirmed by a second PCR using different primers. Two of the four specimens were culture positive in vaginal wash and vaginal swabs, two were culture negative. Recently, a US study evaluated PCR (Amplicor) compared with culture in 95

vaginal specimens from girls being evaluated for suspected sexual abuse.[11] The overall prevalence of *C trachomatis* infection was 12.6%. The specific age of these girls was not given, but the range was 4–16 years, with a mean age of 10.7 years, suggesting that most were probably adolescents, and adolescents have some of the highest rates of *C trachomatis* infection. Nine vaginal specimens were culture and PCR positive, two were culture negative and PCR positive, and one was culture indeterminant and PCR positive, giving a sensitivity of 100% and a specificity of 98%. The positive predictive value (PPV) was 83%. Only one of 30 rectal specimens was PCR and culture positive, one was PCR positive and culture negative and two were PCR negative but culture positive, giving a sensitivity of 33%, specificity of 96% and a PPV of 50%. No discrepant analysis or confirmatory testing was done on the culture negative, PCR positive specimens. These numbers are clearly too small to recommend use of PCR in this setting, especially for rectal specimens.

There are no data on the use of NAAs for detection of *N gonorrhoeae* from either vaginal specimens or urines from prepubertal girls. Although specificity of NAAs may exceed 99%, the adequacy of positive predictive values in populations with a low prevalence of gonorrhoea—for example, 1–3%, has not been fully determined. In one study of the use of the coamplification PCR with genital and urine specimens from of men and women attending STD clinics in the United States, the sensitivities and specificities for detection of *N gonorrhoeae* in urine from males and females compared with culture were 94.4 and 98.5%, and 90% and 95.9%, respectively.[3] The prevalences of gonorrhoea among men and women were 17.4% and 7.8%, respectively. Discrepant specimens were all resolved by repeat PCR testing with a confirmatory 16SrRNA assay. However, another multicentre evaluation from Europe of over 3000 women attending non-sexually transmitted disease clinics where the prevalence of *N gonorrhoeae* was only 0.3%, found only nine positive samples by coamplification PCR.[6] None of the positive PCR results could be confirmed by the16SrRNA PCR.

If one assumes a prevalence of 2% for gonorrhoea and *C trachomatis* in sexually abused children, and sensitivities and specificities of an NAA of urine from women based on published data, PPV of a positive urine NAA would range from 35% when the sensitivity and specificity was 82% and 97%, respectively, to 66%, when the sensitivity and specificity was 97% and 99%, respectively. The PPV is dependent on the specificity and prevalence. Thus, even with a very sensitive and specific test, the PPVs of NAAs may not be adequate for detection of either *C trachomatis* or *N gonorrhoeae* in sexually abused children. The 1998 guidelines for the treatment of sexually transmitted diseases from the US Centers for Disease Control (CDC)[12] suggested that NAAs could be an alternative for detection of *C trachomatis,* if confirmation is available but culture was unavailable. However, all the confirmatory tests are in-house assays and are not commercially available or FDA approved. One could conceivably confirm a positive NAA result with another approved assay, which uses a different genetic target, but most laboratories only use one test. Even in adults, there have been problems with reproducibility of PCR and LCR[13 14] for detection of *C trachomatis* and *N gonorrhoeae*. Although we are concerned about missing possible sexual abuse, it is important to remember that a false positive test for a sexually transmitted disease can lead to erroneous reports of sexual abuse and possibly unjustified prosecution and incarceration. In the absence of a comprehensive, prospective evaluation of NAAs compared with culture for detection of *C trachomatis* and *N gonorrhoeae* in children who are suspected victims of sexual abuse and the lack of commercially available confirmation tests, it would be premature to recommend the use of these assays for this indication at this time.

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Chlamydia trachomatis and cancer

Genital *Chlamydia trachomatis* infections have been recognised as a major public health problem. The World Health Organization (WHO) estimates that 50 million cases of *C trachomatis* infection occur each year worldwide.[1] *C trachomatis* is the major cause of mucopurulent cervicitis, pelvic inflammatory disease, tubal factor infertility, and ectopic pregnancy.[2–5] Thus, the healthcare costs due to complications caused by *C trachomatis* infections are enormous.

Cervical cancer is the most common cancer in women worldwide. Epidemiological studies have shown that early sexual activity is a risk factor for cervical cancer.[6] High risk human papillomavirus (HPV) types are found in practically all cervical carcinomas.[7] The evidence linking oncogenic HPV types in the aetiology of cervical carcinoma is beyond doubt. HPV DNA based longitudinal studies have confirmed the seroepidemiological findings